Original Article

Low expression of GNAI3 predicts poor prognosis in patients with HCC

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Abstract: Purposes: This study was performed with an aim to explain the underlying role of GNAI3 on the prognosis of patients with HCC. Methods: The expression of GNAI3 at protein level was detected with the utilization of Immunohistochemistry (IHC). Chi-square test was conducted to assay the relationship between GNAI3 expression and clinical parameters of HCC patients. The correlation between expression level of GNAI3 and survival time after surgeries of HCC patients was evaluated by Kaplan-Meier method. Finally, the Cox regression was established to evaluate the relationship between GNAI3 expression and the prognosis of patients with HCC. Results: In this study, the negative rate of GNAI3 expression in HCC samples was about 76.6%, which was significantly higher than that in paired normal specimens (12.5%). Result showed that there was no correlation between GNAI3 expression and age, gender, liver cirrhosis and vein invasion (P>0.05), but tight relationship between GNAI3 expression and TNM stage and tumor size was found (P<0.05). The following Kaplan-Meier analysis result illustrated that negative expression of GNAI3 induced high mortality of HCC patients. Cox regression result revealed that GNAI3 might be a biomarker for prognosis of patients with HCC (HR: 0.218, P=0.016, 95% CI 0.063-0.750). Conclusion: Generally, results of this study demonstrated that expression of GNAI3 shared a tight relationship with the prognosis of patients with HCC. Therefore, GNAI3 could be considered as a novel index for prognosis of patients with HCC.

Keywords: HCC, GNAI3, prognosis

Introduction

Liver cancer consists of primary hepatic carcinoma and metastatic hepatic carcinoma. Hepatocellular carcinoma (HCC) accounts for 80% of primary hepatic carcinoma in adults [1-3], which is one of the most common malignancies in Africa and Asia, especially in China and Japan [4-6]. Studies have shown that HCC has an increasing incidence and a poor 5-year survival rate of about 7% despite treatment [7-9]. Currently, therapy of HCC was mainly surgical resection, and sometimes liver transplantation, radiotherapy and some other therapies are also adopted. However, due to advanced disease at the time of diagnosis, lack of suitable organ donors and the influence of radiosensitivity and other factors, the effects of therapies are not obvious [10-14]. Therefore, it is crucial to develop beneficial markers and therapeutic targets for HCC [15].

Guanine nucleotide binding proteins (G-proteins) are a family of signal mediators that are essential for a variety of cellular functions [16]. They widely exist in cells and can function as switches to transduce and regulate signals from outside to inside in the cells [17-19]. Heterotrimeric G protein complexes are typically made up of α, β and γ subunits [20]. The Guanine nucleotide binding protein, alpha inhibiting activity polypeptide 3 (GNAI3) belongs to the α subunit [21], which locates at chromosome hand 17q22-24. GNAI3 has been shown to affect cytokinesis. Recent years, accumulating evidences have demonstrated that GNAI3 is involved in regulating lots of cellular functions such as proliferation, migration, invasion and apoptosis [22-26]. Besides, GNAI3 can produce seemingly paradoxical promotion and inhibition on invasion of different cell lines.

Recently, with the urgent demand to find novel and promising markers for HCC, GNAI3 has
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Table 1. Different expression level of GNAI3 in HCC tissues and paired normal tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Case NO.</th>
<th>Expression</th>
<th>Negative</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCC</td>
<td>64</td>
<td>15</td>
<td>49</td>
<td>76.6%</td>
</tr>
<tr>
<td>Normal</td>
<td>64</td>
<td>8</td>
<td>56</td>
<td>12.5%</td>
</tr>
</tbody>
</table>

Table 2. Association between clinical parameters and GNAI3 expression

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Case number</th>
<th>Protein expression</th>
<th>χ²</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td>Positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>40</td>
<td>10</td>
<td>0.145</td>
<td>0.703</td>
</tr>
<tr>
<td>Female</td>
<td>24</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td>Positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤55</td>
<td>50</td>
<td>12</td>
<td>0.040</td>
<td>0.841</td>
</tr>
<tr>
<td>&gt;55</td>
<td>14</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td></td>
<td>Positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>36</td>
<td>7</td>
<td>0.731</td>
<td>0.393</td>
</tr>
<tr>
<td>No</td>
<td>28</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNM stage</td>
<td></td>
<td>Positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I, II</td>
<td>35</td>
<td>12</td>
<td>5.066</td>
<td>0.024</td>
</tr>
<tr>
<td>III</td>
<td>29</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor size (cm)</td>
<td></td>
<td>Positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤4.5</td>
<td>41</td>
<td>13</td>
<td>4.348</td>
<td>0.037</td>
</tr>
<tr>
<td>&gt;4.5</td>
<td>23</td>
<td>2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Method and materials

Patients and specimens

HCC samples and paired normal specimens used in this study were obtained from 64 pre-operatively untreated patients with histologically confirmed HCC, including 40 males and 24 females, aged from 21 to 65, with a mean age of 37 years, in Zhujiang Hospital. None of the selected patients experienced preoperative cancer treatment. The surgical specimens were cut into small pieces and stored in liquid nitrogen under aseptic condition immediately. This investigation lasted for about 4 years, from April 2010 to May 2014. This retrospective study was approved by our institution’s research ethics board. All patients involved in this study were asked to write an informed consent.

Immunohistochemistry

The expression level of GNAI3 in 64 cases of HCC tissues and paired normal tissues were tested with the utilization of immunohistochemistry (IHC) method. Concretely, the samples were fixed in 3% formaldehyde solution, embedded in paraffin and then cut into 4 μm-thick sections. Then the prepared sections were deparaffinized and rehydrated in a graded series of alcohols after baking at 65°C for 30 min. Following, 0.01 M citrate buffer (pH 6.0) was used to incubate with the sections at 100°C for 15 min, and cooled at room temperature for another 20 min. After that, the primary antibody rabbit anti-GNAI3 was added to the sections and the mixture was incubated at 4°C overnight. Later, we added the Biotin-labeled second antibody to each section, incubating 15 min at room temperature, followed by washing with PBS twice, each for 3 min. Finally, staining signaling was developed using DAB by the avidin-biotin- proxidase method. The sections were air-dried and reserved to use. Positive staining of GNAI3 protein showed mainly in cytoplasm.

Statistical analysis

Data collected in this study was analyzed by SPSS18.0 software (SPSS Inc, USA). The correlation between GNAI3 expression and clinical parameters of patients with HCC was evaluated by Chi-squared test. Kaplan-Meier survival method was adopted to determine survival rates of patients with HCC after operation. Then Cox regression analysis was conducted to evaluate the factors that could influence the prognosis of patients with HCC. We considered statistical significance existed when P value was less than 0.05.

Results

Low GNAI3 protein level in HCC tissues

We next explored the expression of GNAI3 in HCC patients with IHC. The result showed that, among the 64 HCC samples, only 15 (23.4%) specimens were of positive expression, but among those paired normal samples, 56 (87.5%) specimens featured with positive expression of
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Table 3. Multivariate analysis of clinical parameters

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>HR</th>
<th>P value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver cirrhosis</td>
<td>2.274</td>
<td>0.037</td>
<td>1.050-4.925</td>
</tr>
<tr>
<td>Tumor size</td>
<td>7.898</td>
<td>0.001</td>
<td>2.359-26.442</td>
</tr>
<tr>
<td>GNAI3 expression</td>
<td>0.218</td>
<td>0.016</td>
<td>0.063-0.750</td>
</tr>
</tbody>
</table>

GNAI3 (Table 1). The result illustrated that the expression of GNAI3 protein in HCC tissues was significantly lower than that in paired normal tissues (P<0.05).

**Correlation between GNAI3 protein expression and clinical parameters of HCC patients**

Further investigation of relationship between the expression of GNAI3 and the clinical parameters of HCC patients was performed. Chi-square result showed that there was a significant relationship between the expression level of GNAI3 and certain clinical parameters, such as TNM stage, tumor size (P<0.05), but no tightly correlation was found between expression level of GNAI3 and other clinical parameters, likely age, gender, liver cirrhosis and vein invasion (P>0.05) (Table 2).

**Low expression of GNAI3 associated with poor prognosis of HCC patients**

Kaplan-Meier survival analysis was utilized to analyze the correlation between the survival time of patients with HCC and the expression of GNAI3 at protein level. A follow-up of patients with HCC was executed after surgeries, ranging from 1 to 48 months with an average value of 34 months. During the follow-up, 29 (59.2%) of the 49 patients with negative GNAI3 expression died, but of the 15 patients with positive GNAI3 expression, only 3 (20%) died. According to the Kaplan-Meier survival curve, we could conclude that the overall survival rate of patients with negative GNAI3 expression was significantly lower than those with positive GNAI3 expression (Figure 1).

Then we further researched the correlation between expression of GNAI3 and prognosis of patients with HCC by multivariate analysis with the utilization of Cox regression. Data showed in Table 3 demonstrated that negative expression of GNAI3 predicted poor prognosis of patients with HCC, indicating that GNAI3 could be an potential biomarker of prognosis of patients with HCC (HR: 0.218, P=0.016, 95% CI 0.063-0.750) (Table 3).

**Discussion**

HCC is one of the most common malignancies all over the world, which carries a heavy socio-economic burden [27]. Due to the time of diagnose, lack of organ donors, high recurrence and some other reasons, a novel gene therapy was urgently needed. GNAI3 involves in a series of biological processes. Nevertheless, the function of GNAI3 on HCC remains unclear. Therefore, this study was conducted with the aim to evaluate the possibility of GNAI3 as a predictor of HCC.

In this research, we first determined the expression of GNAI3 in patients with HCC at protein level by IHC. According to the result, we found...
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that GNAI3 was always downregulated in HCC tissues compared to paired normal tissues. Besides, Yu Zhang et al. reported that GNAI3 had lower expression in HCC tissues than that in normal tissues, which was consistent with our results.

Based our previous consequence, further investigations were conducted to explore the underlying relationship between GNAI3 expression and clinical parameters of patients with HCC. Statistical significance was found between GNAI3 expression and clinical parameters, such as TNM stage and tumor size (P<0.05), indicating that GNAI3 might be an independent predictor for prognosis of HCC patients.

After that, Kaplan-Meier survival analysis and Cox regression analysis were established to validate our hypothesis. It could be concluded that there was a high mortality in HCC patients with low expression of GNAI3. It was also verified that GNAI3 expression was significantly associated with the prognosis of HCC patients, indicating that it can be regarded as a predictor for prognosis of HCC patients. Low expression of GNAI3 stated poor prognosis.

Because GNAI3 was a newly discovered gene, reports on it were minor. Our research was the first time to explore the prognostic function of GNAI3 on HCC, which could provide theory evidence for further investigations on prognosis of diseases.

Taken together, our findings revealed that GNAI3 expression was significantly lower in HCC tissues compared to paired normal tissues. The result showed that GNAI3 could act as an independent indicator for prognosis of HCC patients. Low expression of GNAI3 could induce poor prognosis.

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Disclosure of conflict of interest

None.

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